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Ultrastructure of Blister Formation in Epidermolysis Bullosa Hereditaria: V. Epidermolysis Bullosa Simplex Localisata Type Weber-Cockayne

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The ultrastructure of epidermolysis bullosa simplex type Weber-Cockayne has as yet not been studied systematically. Therefore biopsies of blisters freshly produced by friction after a hot bath were investigated by electron microscopy in 8 subjects of 6 families. Blister formation was found always to start in the basal layer of the epidermis. At first the cytoplasm with the cell organelles appears to become diluted, then holes develop in the cytoplasm which merge and finally form cytolytic blisters between the dermo-epidermal junction and the nucleus. The cell organelles remain remarkably intact. The cause of blister formation is still unknown, however, no structural abnormality was observed.

Epidermolysis bullosa hereditaria simplex localisata Weber-Cockayne (D-EBS-WC) is an autosomal dominant disorder with a high penetrance and relatively constant expressivity [1,2]. It usually starts in early infancy and occurs mainly, sometimes exclusively, during the warm season [1–5]. Blisters occur on palms, soles, lateral margins of feet, hands, and fingers, and sometimes periungual. The clinical picture is remarkably constant. The blisters heal without scarring or other sequelae [5–7]. Frequently, a rather intense trauma is needed for induction of blisters, and therefore so-called tardive forms often occur. Whether males are really more severely affected than females or merely subject hands and feet more often and more vigorously to mechanical stress is not clear. Many cases have only been diagnosed at military service since the low liability to blistering had not posed any problems before [8–11]. The mechanism of blister formation was still unknown, and histological and electron microscopic investigations have been contradictory [5,6,12–14].

It is still not clear whether the generalized Köbner type and the localized Weber-Cockayne type are independent entities or merely represent a range of mutations in the same gene [4].

PATIENTS

8 patients aged 2–39 yr from 6 families with D-EBS-WC were examined (Fig 1). Blistering has been present in all affected subjects since infancy or early childhood. Within a given family, the clinical picture was relatively constant, though less pronounced trauma seemed to be sufficient for bulla induction in children. None felt severely handicapped. All showed a marked temperature dependence. There was no sex predominance.

MATERIALS AND METHODS

Biopsies of fresh blisters and adjacent clinically normal skin were taken under local anesthesia from 1 or 2 affected members of each family. Since 2 biopsies of spontaneous blisters in 1 patient (1-III-1-ER) as well as a further biopsy of a patient (G.K., fem., 47 yr) not included in this study had only revealed a subcorneal and intracorneal split, respectively, fresh blisters were produced by 10 min rotating friction of a previously not traumatized region of the foot margin after warming the skin by a footbath (37–40° C) and excised after a 15 min interval in all but 1 subject (3-V-1-HD, P116). The biopsies were divided into the normal surrounding skin, the blister margin, and the fullblown blister and processed for electron microscopy: Fixation with 3% glutaraldehyde in 0.1 M cacodylate buffer solution, pH 7.4, partial oxidation with hydrogen peroxide [15], postfixation with 1% osmium tetroxide (Millonig's solution), dehydration in graded ethanols and embedding in epoxy resin (Epon 812). Ultrathin sections were treated with uranyl acetate 5–15% and lead citrate (Reynolds) and examined in electron microscopes Philips EM 400, Siemens Elmiskop Ia and JEOL 100 CX at 60–80 kV and 30–50 μm apertures.

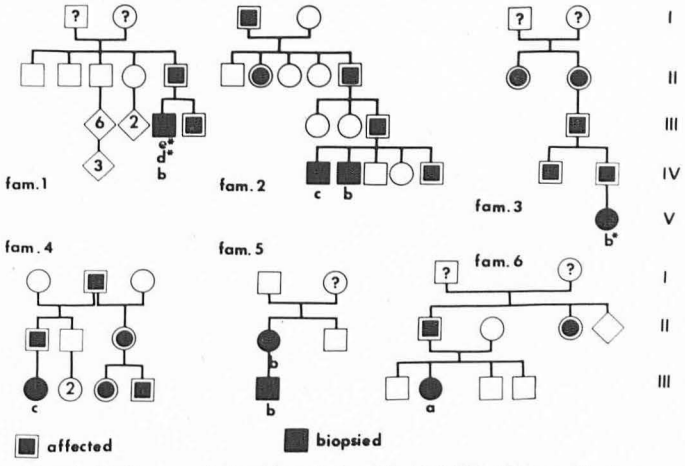

FIG 1. Pedigree of families 1–6 with D-EBS Weber Cockayne. Squares: males, *circles*: females, *upward-standing rectangles*: sex unknown. *Roman numbers* give the generations. Most advanced ultrastructural changes within the biopsies studied: *a*, subnuclear holes in basal cell cytoplasm; *b*, beginning cytolysis; *c*, cytolytic blister; *d*, subcorneal blister; *e*, intracorneal split. *Asterisk*: spontaneous blister.

RESULTS

Light Microscopy

Blisters produced freshly by friction showed a narrow split between almost the entire thickness of the epidermis and the rootlets of the basal cells with the PAS-positive epidermal basement membrane zone. In semithin sections, the bottom of the blisters was stained slightly more intensely with azure II-methylene blue than the rest of the basal cells at the blister roof (Fig 2*a*).

Electron Microscopy

Initial blisters: The initial blister formation started always in the basal layer of the epidermis. The first sign was an edema of the subnuclear cytoplasm with "dilution" of the ribosome-rich cytoplasm (Fig 3*a*). Small holes seemed to appear later in the cytoplasm which then enlarged and merged (Fig 3 *a-d*). In places where such cavities of neighbouring cells reached the

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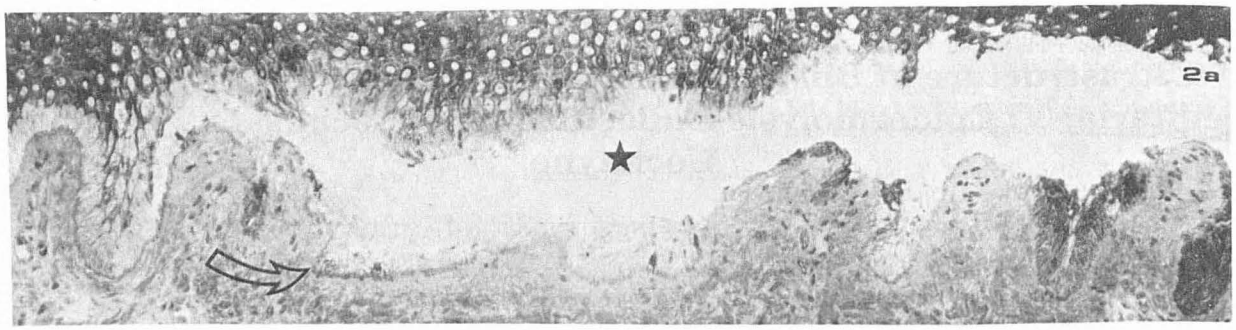
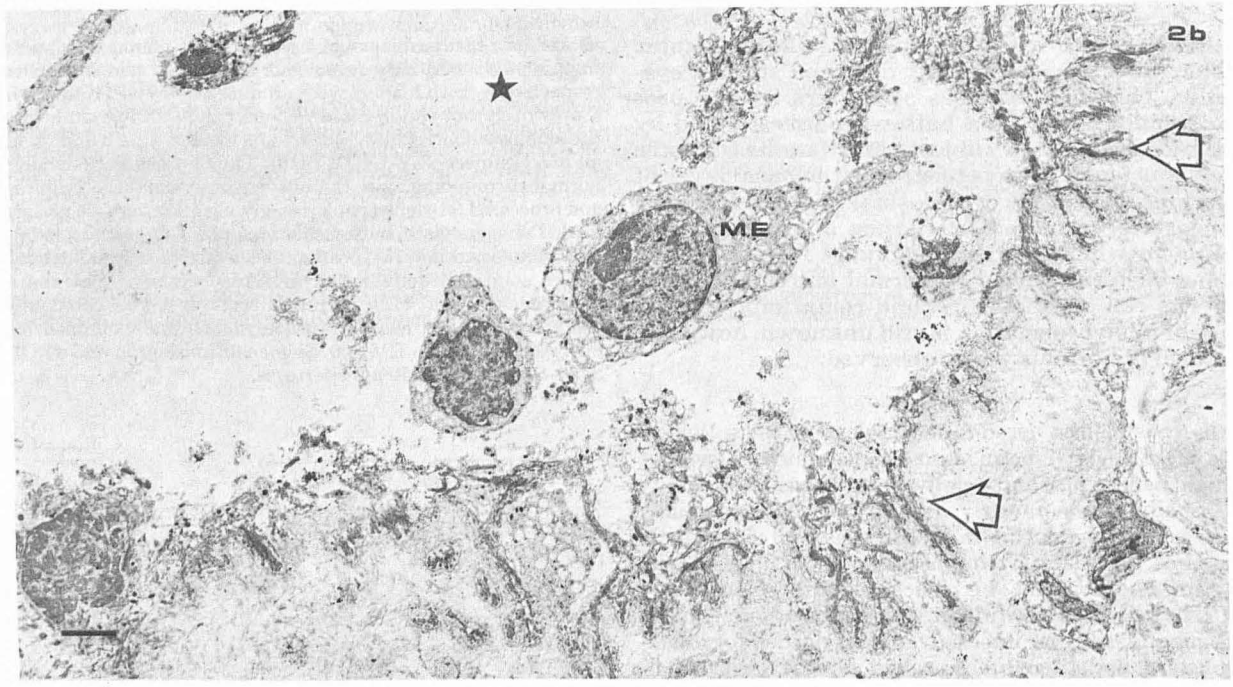
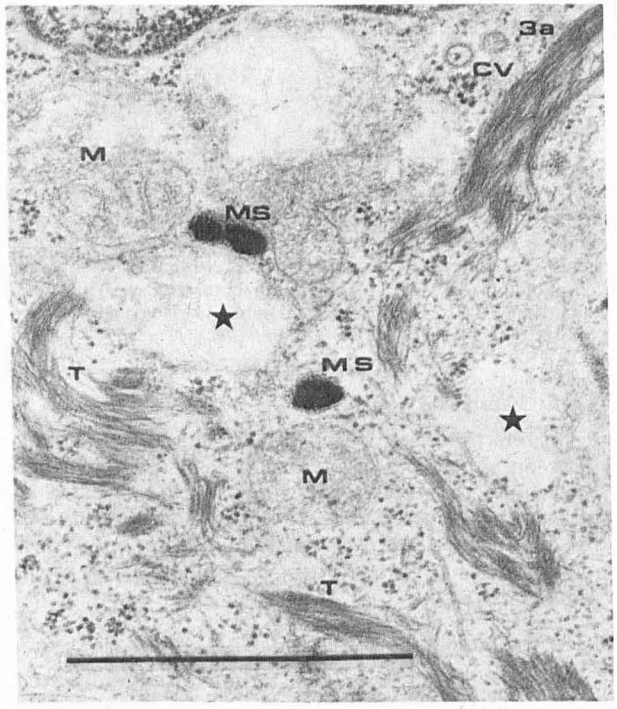
Abbreviations:
D-EBS-WC: epidermolysis bullosa hereditaria localisata Weber-Cockayne







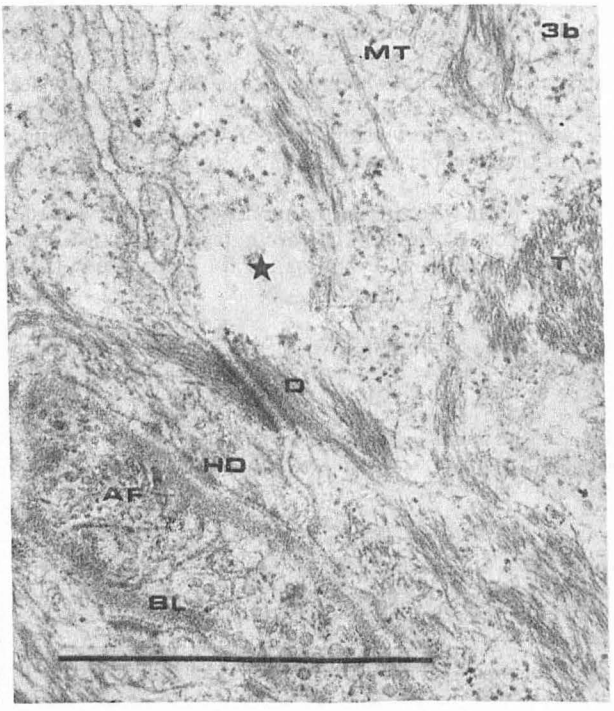


FIG 2. Fresh intraepidermal blister produced by friction; *a*: semithin section (a × 10, azure II-methylene blue), *b*: ultrathin section. *Asterix*: blister cavity; *open arrows*: dermo-epidermal junction at the blister floor; ME-melanocyte floating in the blister fluid (× 7,000).

FIG 3. *a-d*: Initial blister formation via cytolysis in basal cells: Organelles remain unchanged; normal mitochondria (*M*) adjacent to small cytolytic holes (asterix), *D*—desmosomes, *T*—tonofilaments, *MT*—microtubules, CV—coated vesicles, *N*—nucleus, *MS*—melanosomes; AF—anchoring fibrils, BL—basal lamina, HD—hemidesmosomes at the dermo-epidermal junction, *ME*—melanocytic dendrite uninvolved with normal cytoplasm, *E*—erythrocyte in intrabasal split. (*a*, × 45,000, *b*: × 49,000, *c*: × 16,000, *d*: × 137,000).

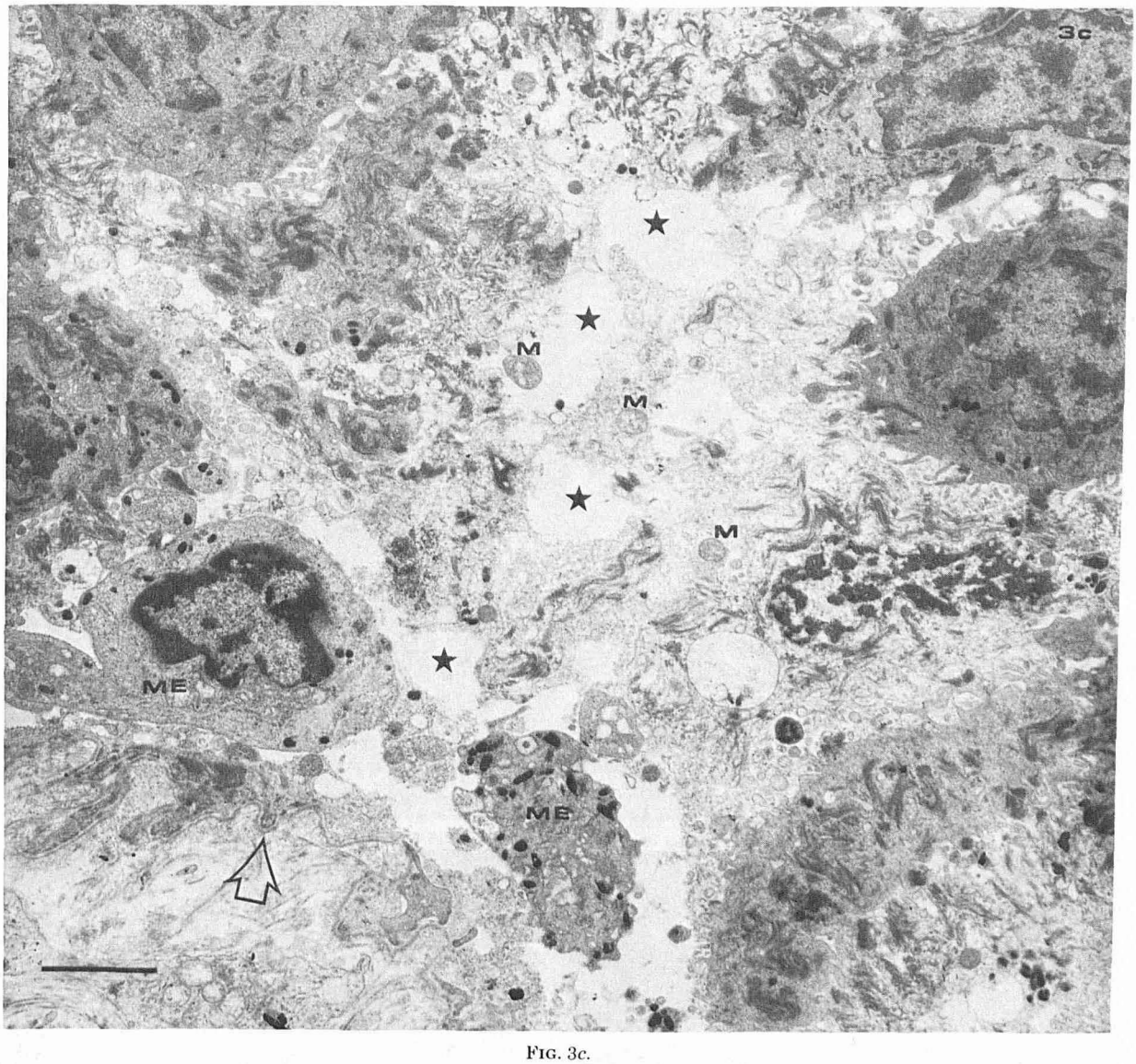


FIG. 3c.

adjacent cell membranes these were ruptured and a true cytolytic blister was present between the lowermost part of the basal cells and the basal nuclei (Fig 2*b*); these stages were already recognizable by light microscopy in semithin sections (Fig 2*a*).

In the initial phase of blister formation, no changes or abnormalities were demonstrable in the different types of cytoplasmic organelles and constituents (Fig 3*a*-3*d*): the tonofilaments remained normal before and during blister formation itself (Fig 3*a*, *b*), and seldom did they appear to condense in fullblown blisters (Fig 4). The mitochondria were intact or only slightly swollen (Fig 3*a*, 3*c*). Rough endoplasmic reticulum, ribosomes, coated vesicles, Golgi apparatus, microtubules, and melanosomes were unchanged (Fig 3*a*, 3*b*). Lysosome-like bodies did not seem to be increased in basal cells. The plasma membranes remained intact during the initial phase and apparently ruptured relatively late (Fig 3*b*). The desmosomes were normal (Fig 3*b*, 3*c*) and could sometimes be observed together with membrane fragments in the blister fluid of fully developed blisters or in the adjacent basal cell cytoplasm (Fig 3*b*). The intercellular space was not enlarged in the initial phase of blister formation (Fig 3*b*, 3*c*).*

Fully developed blisters: The blister floor of friction blisters consisted of the basal cytoplasm of the basal cells and a well-developed dermo-epidermal junction with numerous hemidesmosomes, normal basal lamina, and anchoring fibrils (Fig 2*b*). Melanocytes and Langerhans cells were not involved. Melanocytes within the blister floor, however, were sometimes found floating off into the blister fluid and then showed swelling of the mitochondria and some vacuoles (Fig 2*b*).

The blister roof was formed by the epidermis and the horny layer. The cells were practically unchanged. Even the basal cells torn off from their rootlets did not show marked alterations of their remaining cytoplasm. Only occasionally were the tonofilaments slightly more condensed than normally (Fig 4). A plasma membrane was not found to delimit the remaining cytoplasm of the basal cells from the blister lumen (Fig 5). Seriously damaged pyknotic cells were only very rarely encountered.

Three spontaneously developed blisters biopsied were situated within the horny layer or at the interphase between granular and first horny layer cells. These samples showed a normal basal and spinous layer without any cytolytic changes. The horny cells just above the granular layer were large and showed ruptures of the cell membrane and loose bundles of filaments closely resembling tonofibrils.

DISCUSSION

Light and electron microscopy consistently shows a cytolytic blister formation in the subnuclear region of the basal cells in recently produced fresh bullae. Thus, there is no difference at

* In a control person, friction under the same conditions as used in the EBS-WC patients did not yield a blister nor signs of any other skin damage.

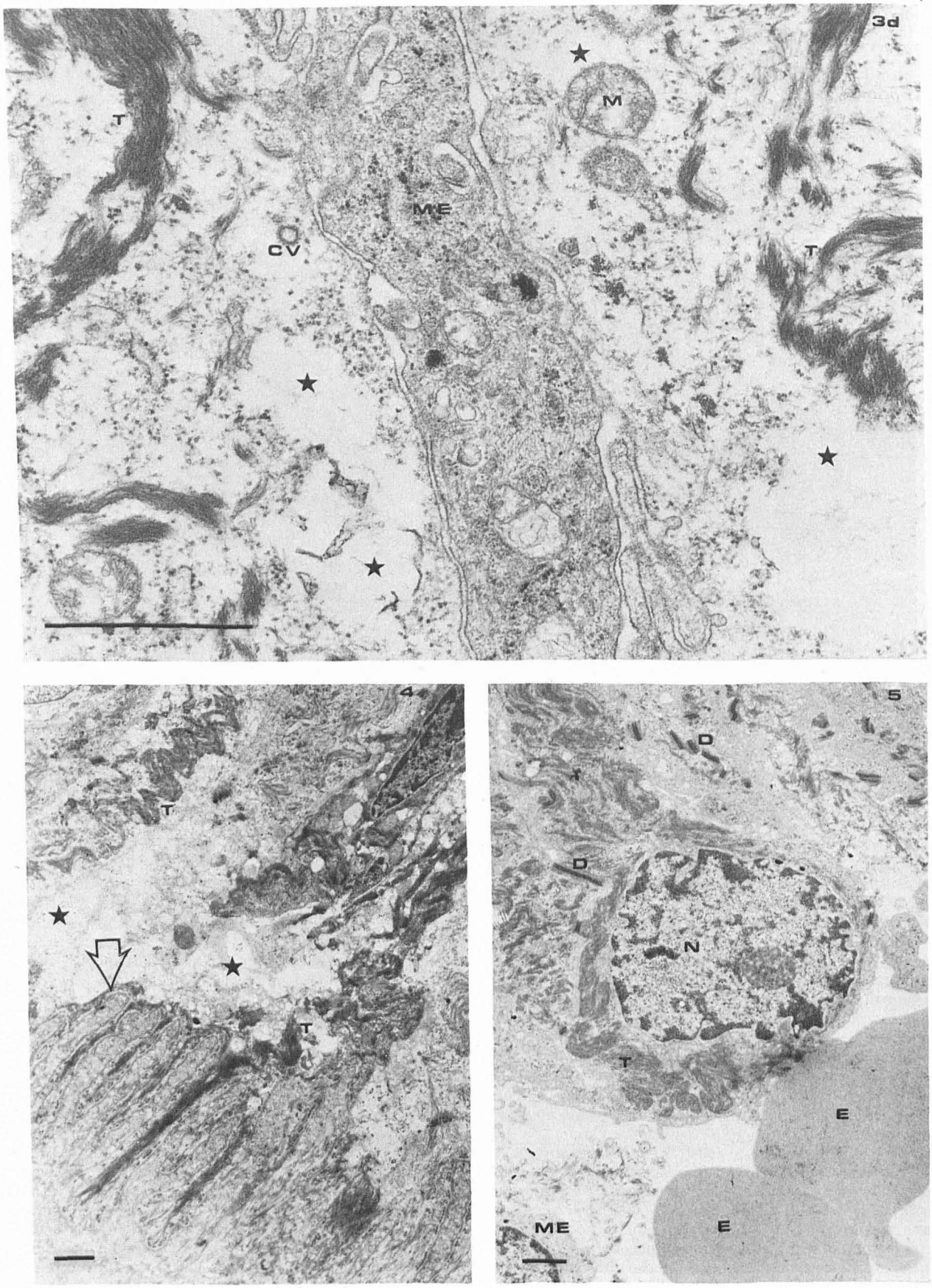


FIG 4. Initial blister formation via cytolysis (*asterix*) above the dermo-epidermal junction (*open arrow*). Tonofilaments (*T*) partly condensed (× 7,000).

FIG 5. Blister roof of fresh blister with almost normal remainder of basal cell; *N*—nucleus, *T*—tonofilaments, and *D*—desmosomes are unchanged. *ME*—melanocyte, and *E*—erythrocytes in the blister fluid (× 8,000).

the ultrastructural level between EBS Weber-Cockayne and EBS Köbner in which identical features are found [5,12,13]. Murata et al [14] recently confirmed these findings. Thus, the Weber-Cockayne and Köbner forms may be genetically related but differ primarily in distribution and therefore in severity. In earlier light [6] and electron microscopic investigations [5], however, the blisters of D-EBS-WC were found to be located in the upper or midstratum spinosum. Because of this similarity with common friction blisters [18] the bulla formation in D-EBS-WC has for a long time been regarded as merely an exaggeration of the normal friction blister mechanism [5,16,17]. In healthy subjects, friction blisters develop from a perinuclear intracellular edema of the Malpighian layer. These changes closely resemble those of minor burns. In contrast to suction blisters, no nuclear deformation is observed [18]. No changes in the stratum spinosum were observed indicating a thermal injury, and all patients tolerated the prewarming procedure. Moreover, melanocytes and Langerhans cells, present within the friction areas, did not reveal any changes or deterioration of their cytoplasm which might have pointed to thermal injury; melanocytes are known generally to be most sensitive among all cell types within the epidermis. Though EB families may vary considerably in the friction time and pressure necessary for blistering a 10 min friction was found to be adequate for our patients to induce early blisters within the biopsy time. Friction was not particularly painful, and patients did not complain of local heat production. However, it must be kept in mind that a humid warm condition is the prerequisite for blister induction. It is therefore felt, that the present findings which differ from previous reports do not represent a burn-type blistering.† Whether these ultrastructural differences point to different subtypes of localized EBS or depend on different biopsy times remains to be elucidated.

We have demonstrated in our patients that experimentally induced fresh blisters in D-EBS-WC always develop in the basal layer with the basal cells remaining surprisingly intact except for their subnuclear splits despite the lack of a plasma membrane present against the lumen border. In contrast, "spontaneous" blisters were found in high levels of the epidermis, and the cytoplasm of the horny cells contained loosely arranged filaments instead of dark staining keratin. Thus it is possible that some sort of repair of the affected epidermal cells might have occurred in subclinical blisters after a "suboptimal" trauma. These cells though injured may have reached the upper epidermal layers and would then be much more liable to rupture upon another trauma such as friction in humid warm condition than are the underlying basal cells so that blisters may appear in the upper epidermis. Sulzberger et al [19] have shown that to produce a blister in normal skin the horny layer must be sufficiently thick and resistent to withstand the frictional forces transmitted downward into the epidermis. The loose, not fully keratinized corneocytes resulting from repair of injured basal cells may not be able to withstand the friction and may therefore rupture. Rotating friction of the margins of the feet or hands after a warm bath yields blisters more constantly than rubbing of the sole itself, if blisters are to be produced in EBS-WC or K.

The mechanism of blister formation in D-EBS-WC is not yet fully understood. The organelles of the basal cells appear to remain intact in the initial phase. It may be speculated that there is a genetically determined temperature-sensitive lability of the gel state of the cytosol, and cytolytic enzymes may also be involved although there is no hint at morphological abnormalities of lysosome-like organelles [13]. There is apparently no other structural anomaly which precedes blister formation. Neither dyskeratotic cells nor clumping of the tonofibrils are found in fresh blisters, and premature cornification of single cells is not observed in older lesions which probably arise as a result of a secondary trauma after a subtotal repair of a primary damage at the basal level.

† In case 3-V-1-HD, P 116, focal cytolysis of basal cells was demonstrated in an area of a spontaneous intracorneal blister without prewarming or frictioning; these changes were indistinguishable from those of experimentally induced initial stages of blister formation.

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